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Impact of the cardiac arrest mode on cardiac death donor lungs

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ABSTRACT

Background: Donation after cardiac death (DCD) organs could alleviate the shortage of donor lungs. This study aimed to assess the influence on lung injuries of the way in which cardiac arrest was induced and to investigate the mechanisms leading to any differences.

Materials and methods: Male rats were allocated into three groups as follows: sham (no warm ischemia), ventricular fibrillation (VF), and asphyxia group. Cardiac arrest was induced by either VF by way of a fibrillator or asphyxia caused by withdrawal of ventilation, which reflected uncontrolled and controlled DCD situations, respectively. The impact on lung flushing after 60 min of warm ischemia time was evaluated ($n = 5$, in each group). The physiological functions of the lungs in an isolated lung perfusion circuit were also evaluated with warm ischemia time prolonged to 150 min ($n = 8$, in each group). Messenger RNA expression levels of surfactant proteins (SPs) and inflammatory cytokines, pathologic findings, and high-energy phosphates of the lung tissues were investigated.

Results: In the asphyxia group, flushing and physiological functions in the isolated lung perfusion circuit were the most severely affected. Reverse transcription-polymerase chain reaction and pathologic findings revealed depletion of surfactant protein (SP)-C in lung tissues of the asphyxia group after reperfusion. The VF group was characteristic with elevated pulmonary vascular resistance.

Conclusions: Lung injuries were mainly attributed to alveolar wall damage and depletion of SP in the asphyxia group, and perivascular area prominent edema in the VF group. DCD donor lungs were affected differently by the way in which cardiac arrest was induced.

1. Introduction

Lung transplantation has been established as the treatment of choice for selected end-stage respiratory diseases; however, a shortage of donor lungs remains a crucial limitation associated with this modality in clinical practice [1,2]. To alleviate this limitation, donor lungs from marginal donors including donation after cardiac death (DCD) donors have been used for lung transplantation [3]. DCD donor lungs are, however, affected by many factors, which differ from the brain death scenario. For instance, premortem hypotension and the mode of death in DCD donors have previously been reported to affect lung functions after reperfusion [4,5], but the influence on lung injury by the way in which cardiac arrest is induced remains unclear.

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In the present study, we hypothesized that during asphyxia in the agonal phase, as seen in a controlled DCD scenario, intra-alveolar oxygen is decreased, which contributes to the pathophysiology of lung injuries. The objectives of this study were therefore to assess the influence on lung injuries of the way in which cardiac arrest is induced and to investigate the mechanisms leading to any differences.

2. Materials and methods

2.1. Animals and types of treatment

Specific pathogen-free inbred male Lewis rats (weight, 290–310 g) were used (Japan SLC Inc, Hamamatsu, Japan). All animals received humane care in accordance with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication 85-23, revised 1996; Bethesda, MD) and the EU directive 2010/63/EU for animal experiments. http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm. The current study protocol was approved by the Graduate School of Medicine Ethical Committee at Kyoto University.

2.2. Statistical analysis

All statistical analyses were performed using the StatView 5.0 software program (Abacus Concepts, Berkeley, CA) on an AT-compatible computer. All values are expressed as mean ± standard deviation. The data were evaluated using one-way analysis of variance, and Scheffe *post hoc* multiple comparison test for multiple group analysis. A probability (P) value <0.05 was considered to be statistically significant.

2.3. Experiment 1

2.3.1. Experimental protocol

The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg), intubated after tracheotomy, and ventilated with room air under the following conditions: tidal volume of 0.01 mL/g; respiratory rate of 60 cycles/min; positive end-expiratory pressure of 3 cm H_2O; and a ratio of inspiratory duration of 50%. The animals were not heparinized. The animals were then randomly allocated into three groups as follows: sham, ventricular fibrillation (VF), and asphyxia groups ($n = 5$ each).

In the sham group, the pulmonary artery was directly cannulated while the hearts were kept beating. In the VF group, VF was induced and continued for 5 min by way of a fibrillator attached directly to the right atrium and apex of the heart meanwhile lungs were ventilated with room air, and then the ventilator was disconnected. In the asphyxia group, the ventilator was disconnected while the tracheal tube was kept open to induce asphyxia. After cardiac arrest was confirmed, the chest was closed with skin staplers, and the rats were placed at room temperature for 60 min. The pulmonary artery was then cannulated, and the lungs were flushed with 50 mL of Trypan blue (Sigma Chemical Co, St. Louise, MO) solution dissolved in normal saline (0.2 mM, 4°C) at a pressure of 20 cm H_2O, and drained through a left atrium incision. The time required for the flushing of each lung was measured, and the gross appearance of lungs dyed with Trypan blue was also evaluated.

2.4. Experiment 2

2.4.1. Experimental protocol

The animals were randomly allocated into sham, VF, and asphyxia groups as described in experiment 1 ($n = 8$ each). The animals were not heparinized. In the sham group, the pulmonary artery was cannulated while the heart was beating and flushed with 20 mL of 4°C low potassium dextran solution (Perfadex; Vitrolife, Uppsala, Sweden) at a pressure of 20 cm H_2O. In the VF and asphyxia groups, animals were placed at room temperature for 150 min after cardiac arrest was confirmed. The pulmonary artery was then cannulated, and the lungs were flushed in the same way as in the sham group.

Ventilation with room air was initiated 5 min before flushing under the following conditions: positive pressure-control mode of +8 and +4 cm H_2O; respiratory rate, 60 cycles/min; and ratio of inspiratory duration of 50%. After flushing, the total lung capacity maneuver using 30 cm of H_2O for 1 min was performed [6]. Heart–lung blocks were then extracted from the animals (Fig. 1).

2.5. Reperfusion in an isolated lung perfusion circuit

Reperfusion of the rat lungs was performed in an isolated rat lung perfusion circuit (Model 829; Hugo-Sachs Elektronik Harvard Apparatus; March-Hugstetten, Germany) as reported previously [7–9]. The perfusate was heparinized rat blood obtained from two donor rats diluted with saline containing 4% bovine serum albumin and was delivered by two pumps. No leukocyte filter was used, and the circuit was water-jacketed to maintain the temperature at 37°C.

The effluent perfusate from the left atrium was deoxygenated in a glass deoxygenator with anoxic gas (nitrogen, 92%; carbon dioxide, 8%) and was pumped into the pulmonary artery. The perfusate was stabilized by circulating outside of

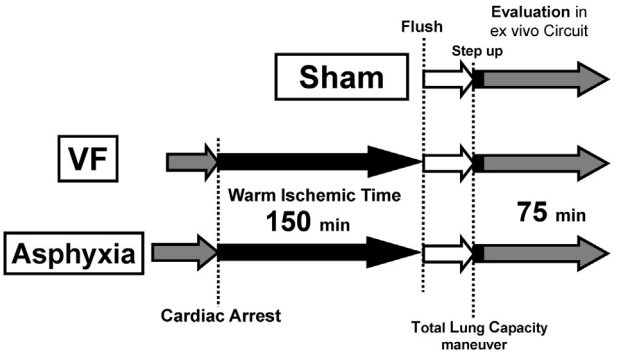

Fig. 1 – The protocol for experiment 2. In the VF and asphyxia groups, after introduction of cardiac arrest, lungs were left *in vivo* for 150 min as the warm ischemic time. A total lung capacity maneuver was performed before the extraction of the heart–lung block. After 5 min for a step-up period, perfusion at 10 mL/min was continued for 75 min in an *ex vivo* lung perfusion circuit.